



US005438017A

United States Patent [19]

Allen et al.

[11] **Patent Number:** **5,438,017**[45] **Date of Patent:** **Aug. 1, 1995**

[54] **ASSAYS FOR SULFHYDRYL AMINO ACIDS AND METHYLMALONIC ACID AND THEIR APPLICATION TO DIAGNOSIS OF COBALAMIN DEFICIENCY**

[75] **Inventors:** Robert H. Allen, Englewood; Sally P. Stabler, Denver, both of Colo.

[73] **Assignee:** The University of Colorado Foundation, Inc., Colo.

[21] **Appl. No.:** 960,783

[22] **Filed:** Oct. 14, 1992

Related U.S. Application Data

[63] Continuation of Ser. No. 345,885, May 1, 1989, abandoned.

[51] **Int. Cl.⁶** G01N 24/00; C12Q 1/00

[52] **U.S. Cl.** 436/89; 436/174; 436/825; 436/86; 436/120; 436/4; 436/18

[58] **Field of Search** 435/4, 18; 436/173-174, 8, 86, 120, 825, 89; 514/52, 258

[56] **References Cited****U.S. PATENT DOCUMENTS**

4,940,658 7/1990 Allen et al. 436/120

OTHER PUBLICATIONS

Information Disclosure Statement submitted in parent application Ser. No. 345,885 dated Nov. 29, 1990.

Declaration under 37 CFR §1.132 dated Nov. 27, 1990, including Exhibit A thereto.

Adams, R. F., "Determination of Amino Acid Profiles in Biological Samples by Gas Chromatography," J. Chromatography (1974) 95:189-212.

Adams, R. F. et al., "Ultramicro GC Determination of Amino Acids Using Glass Open Tubular Columns and a Nitrogen-Selective Detector*", J. Chromatographic Sci. (1977) 15:63-68.

Alberts et al. (1989) Molec. Biol. of the Cell, pp. 583-584, 722-723.

Butler, M. et al., "Amino Acids Attached to Transfer Ribonucleic Acid *in vivo*," Biochem J. (1975) 150:419-432.

Canclon, P. and Klingman, J. D., "An Improved Procedure for Preparing the n-Butyl-Trifluoroacetyl

Amino Acid Derivatives and Its Application in the Study of Radioactive Amino Acids from Biological Sources," J. Chromatographic Science (1974) 12:349-355.

Chauhan, J. and Darbre, A., "Determination of Urinary Amino Acids by Means of Glass Capillary Gas-Liquid Chromatography with Alkali-Flame Ionisation Detection and Flame Ionisation Detection," J. Chromatography (1982) 227:305-321.

Collins, F. S. and Summer, G. K., "Determination of glutamine and glutamic acid in biological fluids by gas chromatography," J. Chromatography (1978) 145:456-463.

Davis et al. (1990) Microbiology, p. 94.

Desgres, J. et al., "Gas-liquid chromatography of isobutyl ester, N(O)-Heptafluorobutyrate derivatives of amino acids on a glass capillary column for quantitative separation in clinical biology," J. Chromatography (1979) 162:133-152.

Doussin, A. and Bourdon, R., "Determination of various amino acids by gas chromatography. Application to blood microsamples absorbed onto paper," Ann Biol. Clin (Paris) (1972) 30(5):417-426 (abstract only).

Faull, K. F. et al., "Mass spectrometric identification and selected ion monitoring quantitation of γ -amino-butyric acid (GABA) in human lumbar cerebrospinal fluid," J. Neurochemistry (1978) 31:1119-1122.

(List continued on next page.)

Primary Examiner—Lyle A. Alexander

Attorney, Agent, or Firm—Greenlee and Winner

[57]

ABSTRACT

Improved methods for the GC/MS analysis of sulfhydryl amino acids and methylmalonic acids in samples of body fluids are provided. Additionally a method for combined assay of sulfhydryl amino acids particularly total homocysteine and combined creatine/creatinine levels in samples of body fluids provided. The information provided by the methods described is useful in the detection of the presence of cobalamin or folate deficiencies in individuals and in distinguishing between the two deficiencies.

22 Claims, No Drawings